

FORM PTO-1390 (Modified) (REV. 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				21508-033
INTERNATIONAL APPLICATION NO. PCT/US99/07745		INTERNATIONAL FILING DATE 08 April 1999 (08.04.99)		U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR 09/937735
TITLE OF INVENTION INDUCTION OF KIDNEY TUBULE FORMATION		PRIORITY DATE CLAIMED		
APPLICANT(S) FOR DO/EO/US MCMAHON, Andrew P.; KISPERT, Andreas; VAINIO, Seppo.				
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:				
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below. 4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2)) <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> has been communicated by the International Bureau c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto. b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4) 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). 10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)). 11. <input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409). 12. <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210) 				
Items 13 to 20 below concern document(s) or information included: <ol style="list-style-type: none"> 13. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 15. <input type="checkbox"/> A FIRST preliminary amendment. 16. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 17. <input type="checkbox"/> A substitute specification. 18. <input type="checkbox"/> A change of power of attorney and/or address letter. 19. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. 20. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). 21. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 22. <input checked="" type="checkbox"/> Certificate of Mailing by Express Mail 23. <input checked="" type="checkbox"/> Other items or information: 				
Express Mail Label No: EL390884359US Filed On: 28 September 2001 (28.09.01)				

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR) 09/937735	INTERNATIONAL APPLICATION NO. PCT/US99/07745	ATTORNEY'S DOCKET NUMBER 21508-033																
24. The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		CALCULATIONS PTO USE ONLY																
<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1000.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00																		
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$100.00																
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). 		\$130.00																
<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 25%;">CLAIMS</th> <th style="width: 25%;">NUMBER FILED</th> <th style="width: 25%;">NUMBER EXTRA</th> <th style="width: 25%;">RATE</th> </tr> </thead> <tbody> <tr> <td>Total claims</td> <td>21 - 20 =</td> <td>1</td> <td>x \$18.00 \$18.00</td> </tr> <tr> <td>Independent claims</td> <td>3 - 3 =</td> <td>0</td> <td>x \$80.00 \$0.00</td> </tr> <tr> <td colspan="3">Multiple Dependent Claims (check if applicable).</td> <td style="text-align: center;"><input type="checkbox"/> \$0.00</td> </tr> </tbody> </table>		CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	Total claims	21 - 20 =	1	x \$18.00 \$18.00	Independent claims	3 - 3 =	0	x \$80.00 \$0.00	Multiple Dependent Claims (check if applicable).			<input type="checkbox"/> \$0.00	
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TOTAL OF ABOVE CALCULATIONS =		\$248.00																
<input type="checkbox"/> Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.		\$0.00																
SUBTOTAL =		\$248.00																
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). 		\$0.00																
TOTAL NATIONAL FEE =		\$248.00																
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).		<input type="checkbox"/> \$0.00																
TOTAL FEES ENCLOSED =		\$248.00																
		Amount to be: refunded \$ charged \$																
a. <input checked="" type="checkbox"/> A check in the amount of \$248.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-0311 A duplicate copy of this sheet is enclosed. d. <input type="checkbox"/> Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.																		
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.																		
SEND ALL CORRESPONDENCE TO. <div style="border: 1px solid black; padding: 5px; display: inline-block;"> BEATTIE, Ingrid A. Mintz, Levin, Cohn, Ferris, Glovsky & Popeo, P.C. One Financial Center Boston, Massachusetts 02111 United States of America </div>																		
 SIGNATURE Ingrid A. Beattie NAME 42,306 REGISTRATION NUMBER 28 September 2001 (28.09.01) DATE																		

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS : McMahon *et al.*
SERIAL NUMBER : 09/937,735 EXAMINER : Not Yet Assigned
FILING DATE : Sept 28, 2001 ART UNIT : Not Yet Assigned
FOR : **INDUCTION OF KIDNEY TUBULE FORMATION**

April 29, 2002
Boston, Massachusetts

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P.O. 2327
Arlington, VA 22202

PRELIMINARY AMENDMENT

Prior to examination of the above-identified patent application, please amend the application as set forth below and consider the following remarks.

In the Specification:

Please insert the Sequence Listing, pages 1-10, at the end of the specification.

REMARKS

Applicants submit a Sequence Listing for the nucleotide sequences disclosed in the specification, in compliance with the requirements for patent applications containing nucleotide sequences and/or amino acid sequence disclosures. 37 C.F.R. §§ 1.821-1.825.

CONCLUSION

Applicants respectfully submit that the present application complies with 37 C.F.R. §§ 1.821-1.825. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,


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WO 00/61630

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09/937735

INDUCTION OF KIDNEY TUBULE FORMATION

Statement as to Federally Sponsored Research

5 This invention was funded in part by the U.S. Government under grant number HD30249 awarded by the National Institutes of Health. The Government has certain rights in the invention.

Background of the Invention

10 Kidney and urinary tract diseases are major causes of illness and death in the United States resulting in about 50,000 deaths per year. Renal cell carcinoma is the most common type of kidney cancer; this type of cancer affects the lining of the renal tubule and is
15 often metastatic. About one third of the cases diagnosed show metastasis, e.g., to the lung or other organs, at the time of diagnosis. Other types of medical conditions, such as diabetes mellitus and high blood pressure, can lead to chronic kidney failure.
20 Current therapeutic approaches include dialysis and transplantation.

Summary of the Invention

The invention provides a method of regenerating kidney tissue and is based on the discovery that Wnt-4 is
25 sufficient to trigger kidney tubulogenesis, whereas Wnt-11 (which is also involved in tubule formation) is not. Kidney tubule formation in a post-natal mammal is stimulated by administering to the mammal a substantially pure Wnt polypeptide or a Wnt agonist. Preferably, the
30 Wnt polypeptide is Wnt-4 or a Wnt-1 class polypeptide such as Wnt-1, Wnt-2, Wnt-3a, Wnt-7a, and Wnt-7b. A Wnt-1 class polypeptide is a Wnt polypeptide that transforms C57MG cells in culture. More preferably, the polypeptide is Wnt-3a, Wnt-4, Wnt-7a, and Wnt-7b, but not members of
35 the Wnt-5a class of proteins such as Wnt-5a or Wnt-11. For example, the Wnt polypeptide is Wnt-4, and the Wnt

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agonist is HLDAT86. Wnt-4 mediated-tubulogenesis requires cell contact; accordingly, Wnt compositions are preferably administered to kidney cells in the context of the kidney organ or in a situation in which the cells 5 expressing a Wnt polypeptide or agonist are in close contact with cells involved in tubule formation. In preferred embodiments, sulfated glycosaminoglycans (sGAGs) are co-administered with the Wnt compositions.

The mammal to be treated is characterized as 10 suffering from a kidney disorder. Preferably, the mammal is a human, mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, monkey, dog, or cat. The method is therapeutic or preventative and is administered to a juvenile or adult mammal. Kidney disorders include 15 chronic renal failure, renal cell carcinoma, polycystic kidney disease, chronic obstructive uropathy, and virus-induced nephropathy. For example, the method is used to treat or prevent renal tubule epithelial cell degeneration associated with HIV-1 infection.

20 Administration of the Wnt compositions is local or systemic. For example, the polypeptide or Wnt agonist is administered locally to a renal tissue by, e.g., retrograde perfusion of renal tissue via blood vessels or urine collecting ducts. Wnt compositions are also 25 administered *ex vivo* to an explanted renal tissue. For example, a kidney is removed from an individual and treated *in vitro* with a Wnt composition (e.g., a substantially pure polypeptide or an isolated nucleic acid) and then returned to the body of the same 30 individual or a different individual.

The Wnt composition is a peptide mimetic, e.g., a polypeptide that is more resistant to proteolytic cleavage compared to a naturally-occurring Wnt polypeptide. The Wnt polypeptide is preferably soluble 35 under physiological conditions. Accordingly, the

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polypeptide is modified to improve its solubility. Alternatively, the Wnt polypeptide is present on the surface of a cell. The method utilizes a Wnt polypeptide that includes an amino acid sequence that is at least 85% 5 identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5, a Wnt polypeptide that includes an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5, a Wnt polypeptide that includes an amino acid sequence that is at least 95% 10 identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5, and a Wnt polypeptide that includes an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5. The Wnt polypeptide preferably has an amino acid sequence at 15 least 85% identical to SEQ ID NO: and functions to stimulate tubulogenesis. For example, the polypeptide may be a fragment of Wnt that stimulates tubulogenesis. A fragment has an amino acid sequence that is identical to part, but not all, of the amino acid sequence of a 20 naturally-occurring Wnt polypeptide. A fragment of a naturally-occurring Wnt polypeptide that stimulates tubulogenesis preferably includes the amino acid sequence of at least the amino-terminal 50% of the naturally-occurring polypeptide. More preferably, the fragment 25 contains the amino acid sequence of at least the amino terminal 75% of a naturally-occurring Wnt polypeptide. For example, the fragment contains at least residues 1- 180 of naturally-occurring Wnt-1 (SEQ ID NO:1). Other fragments of Wnt polypeptides which have been shown to 30 stimulate tubulogenesis, e.g., residues 100-331 of naturally-occurring Wnt-7a (SEQ ID NO:4, highlighted in bold), are administered. Full-length Wnt polypeptides or fragments thereof are chemically or recombinantly linked to Ig to yield Wnt-Ig fusion proteins. Human or mouse 35 Wnt polypeptides are administered to mammals to stimulate

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tubulogenesis.

Also within the invention is a method of stimulating kidney tubule formation in a post-natal mammal by administering a substantially pure or isolated 5 nucleic acid encoding a Wnt polypeptide (e.g., a nucleic acid having the nucleotide sequence of SEQ ID NO:10, 11, or 12) or a Wnt agonist. Nucleic acids that encode a Wnt polypeptide and that have a sequence that is substantially identical to a Wnt-encoding nucleic acid 10 sequence are administered to diseased kidney tissue.

Polypeptides or other compounds of interest are said to be "substantially pure" when they are within preparations that are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is 15 at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

20 A polypeptide or nucleic acid molecule which is "substantially identical" to a given reference polypeptide or nucleic acid molecule is a polypeptide or nucleic acid molecule having a sequence that has at least 85%, preferably 90%, and more preferably 95%, 98%, 99% or 25 more identity to the sequence of the given reference polypeptide sequence or nucleic acid molecule.

"Identity" has an art-recognized meaning and is calculated using well known published techniques, e.g., Computational Molecular Biology, 1988, Lesk A.M., ed., 30 Oxford University Press, New York; Biocomputing: Informatics and Genome Projects, 1993, Smith, D.W., ed., Academic Press, New York; Computer Analysis of Sequence Data, Part I, 1994, Griffin, A.M. and Griffin, H.G., eds, Humana Press, New Jersey; Sequence Analysis in Molecular 35 Biology, 1987, Heinje, G., Academic Press, New York; and

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Sequence Analysis Primer, 1991, Gribskov, M. and Devereux, J., eds., Stockton Press, New York). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the 5 term "identity" is well known to skilled artisans and has a definite meaning with respect to a given specified method. Sequence identity is measured using the Sequence Analysis Software Package of the Genetics Computer Group (GCS), University of Wisconsin Biotechnology Center, 1710 10 University Avenue, Madison, WI 53705), with the default parameters as specified therein.

By "isolated nucleic acid molecule" is meant a nucleic acid molecule that is free of the genes which, in the naturally-occurring genome of the organism, flank a 15 gene encoding a Wnt polypeptide. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate 20 molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence such as an immunoglobulin 25 polypeptide. The term excludes large segments of genomic DNA, e.g., such as those present in cosmid clones, which contain a gene of interest flanked by one or more other genes which naturally flank it in a naturally-occurring genome.

30 Nucleic acid molecules include both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. Where single-stranded, the nucleic acid molecule may be a sense strand or an antisense strand. The term therefore includes, for 35 example, a recombinant DNA which is incorporated into a

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vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote at a site other than its natural site; or which exists as a separate molecule (e.g., a cDNA or a genomic 5 or cDNA fragment produced by polymerase chain reaction (PCR) or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence such as an Ig polypeptide.

10 Wnt nucleic acids (encoding Wnt polypeptides) which hybridize at high stringency to naturally-occurring Wnt-encoding sequences are also administered to stimulate tubulogenesis. Hybridization is carried out using standard techniques such as those described in Ausubel et 15 al., *Current Protocols in Molecular Biology*, John Wiley & Sons, (1989). "High stringency" refers to DNA hybridization and wash conditions characterized by high temperature and low salt concentration, e.g., wash conditions of 65° C at a salt concentration of 20 approximately 0.1 X SSC. "Low" to "moderate" stringency refers to DNA hybridization and wash conditions characterized by low temperature and high salt concentration, e.g. wash conditions of less than 60° C at a salt concentration of at least 1.0 X SSC. For example, 25 high stringency conditions may include hybridization at about 42°C, and about 50% formamide; a first wash at about 65°C, about 2X SSC, and 1% SDS; followed by a second wash at about 65°C and about 0.1% x SSC. Lower stringency conditions suitable for detecting DNA 30 sequences having about 50% sequence identity to *csa-1* gene are detected by, for example, hybridization at about 42°C in the absence of formamide; a first wash at about 42°C, about 6X SSC, and about 1% SDS; and a second wash at about 50°C, about 6X SSC, and about 1% SDS.

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The invention also includes an *ex vivo* mammalian kidney containing an exogenous Wnt polypeptide, e.g., having been bathed in or perfused with a solution containing a Wnt polypeptide or agonist. Alternatively, 5 the *ex vivo* mammalian kidney contains exogenous DNA encoding a Wnt polypeptide. The kidney is bathed or perfused with a solution containing a Wnt-encoding nucleic acid, and cells of the kidney take up the DNA. The cells then express and secrete the recombinant Wnt 10 polypeptide or agonist. For expression of recombinant Wnt polypeptides, Wnt-encoding sequences are operably linked to regulatory sequences, e.g., tissue specific promoters. Kidney-specific promoters are known in the art and include, e.g., the Pax-2 promoter, the cRET 15 promoter, and the Hox b7 promoter. By "operably linked" is meant able to promote transcription of an mRNA corresponding to a polypeptide-encoding sequence located downstream on the same DNA strand.

Description of the Preferred Embodiments

20 A Wnt polypeptide, e.g., Wnt-4, Wnt-1, Wnt-3a, Wnt-7a and Wnt-7b, acts as a trigger to start an intrinsic program in the mesenchymal cells which then proceed to form complex nephron like structures. Wnt-4 is a secreted glycoprotein which is required for kidney 25 tubule formation. Development of the mammalian kidney is initiated by ingrowth of the ureteric bud into the metanephric blastema. In response to signal(s) from the ureter, mesenchymal cells condense, aggregate into pretubular clusters, and undergo epithelialisation to 30 form simple epithelial tubules. Subsequent morphogenesis and differentiation of the tubular epithelium lead to the establishment of a functional nephron.

Table 6: Human Wnt-1 amino acid sequence

1 MGLWALLPGW VSATLLALA ALPAALAANS SGRWWGIVNV ASSTNLLTDS
35 KSLQLVLEPS

- 8 -

61 LQLLSRKQRR LIRQNPGLIH SVSGGLQSAV RECKWQFRNR RWNCPTAPGP
HLFGKIVNRG

121 CRETAFIFAI TSAGVTHSVA RSCSEGSIES CTCDYRRRGP GGPDWHWGGC
SDNIDFGRLF

5 181 GREFVDSGEK GRDLRFLMNL HNNEAGRRTV FSEMROECKC HGMGSCTVR
TCWMRLPTLR

241 AVGDVLRDRF DGASRVLYGN RGSNRASRAE LLRLEPEDPA HKPPSPHDLV
YFEKSPNFCT

301 YSGRLGTAGT AGRACNSSSP ALDGCELLCC GRGHRTRTQR VTERCNCTFH

10 WCCHVSCRNC

361 THTRVLHECL (SEQ ID NO:1)

Table 7: Human Wnt-3a amino acid sequence

CKCHGLSGSC EVKTCWWSQP DFRAIGDFLK DKYDSASEMV VEKHRESRGW
VETLRPRYTY FKVPTERDLV YYEASPNFCE PNPETGSFGT RDRTCNVSSH

15 GIDGCDLLCC GRGHNARAER RREKRCVFH WCC (SEQ ID NO:2)

Table 8: Human Wnt-4 amino acid sequence

CKCH GVSGSCEVKT CWRAVPPFRQ VGHALKEKFD GATEVEPRRV GSSRALVPRN AQFKPHTDED
LVYLEPSPDF CEQDMRSGVL GTRGRTCNKT SKAIDGCELL CCGRGFHTAQ

VELAERCSCCK

20 FHWCLFLSR (SEQ ID NO:3)

Table 9: Human Wnt-7a amino acid sequence

1 MNRKALRCLG HLFLSLGMVC LRIGGFSSVV ALGATIICNK IPGLAPRQRA ICQSRPDAII
61 VIGEGSQMGL DECQFQFRNG RWNCSALGER TVFGKELKVG SRDGAFTYAI IAAGVAHAI
121 AACTHGNLSD CGCDKEKQGQ YHRDEGWKNG GCSADIRYGI GFAKVFVDAR EIKQNARTLM

25 181 NLHNNEAGRK ILEENMKLEC KCHGVSGSCT TKTCTTLPQ FRELGYVLKD KYNEAVHVEP
241 VRASRNKRPT FLKIKKPLSY RKPMMDTLVY IEKSPNYCEE DPVTGSVGTQ GRACNKTAPQ
301 ASGCDLMCCG RGYNTHQYAR VVQCNCKFHW CCYVKCNTCS ERTEMYTCK
(SEQ ID NO:4)

Table 10: Human Wnt-7b partial amino acid sequence

30 VKC GVSGSCTTKT CWTTLPKFRE VGHLLKEKYN AAVQVEVVRA SRLRQPTFLR IKQLRSYQKP
METDLVYIEK SPNYCEEDAA TGSVGTQGRI CNRTSPGADG CDTMCCGRGY NTHQYTKVWQ
CNCKFHWCCS (SEQ ID NO:5)

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Table 11: Human Wnt-1 Nucleotide Sequence

1 atgtatgtat gtatgtatgt atgtatgtat acgtgcgtgc acctgtgtgt
 gcttgggtgtc
 61 agtggggctc agacatcacc tgattccctg gaactggagt tacaggtggc
 5 tataagccac
 121 cacttgggtg ctgagaacag agtccgggccc tctggcagag cagtcagtgc
 ttttagccac
 181 tgagccactc tcatcccccc aattatgttc atctttagttt gggcaggtac
 ggtggcggaa
 10 241 taggcctgta atcccagcag tcactggacc atcatggttt ctacatatttt
 aacctttatg
 301 ttaggttaggg tcacacagca agatccggtc acaaaaaccag caacaacaaa
 aaccaaaaagg
 361 agccagcttc ttcccacaag cattttcc ctcaggtctt cagctccatc
 15 tgacagactac
 421 tggctggtg gtcctatcct ttctgagcct agttgccaga gaaacaagcc
 cggttcatct
 481 tcactgactag cacatctaata gataaggcaca ggttgactca aggtgccata
 gagtgacact
 20 541 aggtacccag agcgacagaa tgacacctat gagtgcacgt cgttaatcac
 aaacacacac
 601 acacacacac acacacacac acacacacac tcacgcaccc acctgcaaac
 acaattgcag
 661 cttctggac gtctctgtc acagccccac ctcttcctg atacactgct
 25 ttaagtggtg
 721 actgtaaaca aatgacttca tgctctccct gtcctgagcc aaattacaca
 attatttggaa
 781 aagggtctcaa aatgttcttc gttagaagtt tctggataca ccaatacaca
 ggagcgtgca
 30 841 ccctcagaac acatgtacac tttgacttaa tctcacgggt gacacaccga
 cgcttacact
 901 ccccttagcc cacagaggca aactgctggg cgcttctgag tttctcactg
 ccaccagctc
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 35 gtcacccca
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 tgaggaagtg
 1081 tctccacgga gtcgctggct agaaccacaa ctttcattcct gccattcaga
 atagggaaaga
 40 1141 gaagagacca cagcgttaggg gggacagagg agacggactt cgagaggaca
 gccccacccgg
 1201 cgcgtgtggg ggaggcaatc caggctgcaa acaggttgct cccagcgtcat
 tgcggccgg

- 10 -

1261 cccccctggcg gatgctggc cccgacgggc tccggacgac cagaagagtg
aggccggcgc
1321 gcgtgggagg ccatccaaag gggaggggtc ggccggccagt gcagacactgg
aggcggggcc
5 1381 accaggcagg gggcgggggt gagccccac gtttagcctg tcagctctt
gctcagaccg
1441 gcaagagcca cagcttcgct cgccactcat tgctctgtggc cctgaccagt
gcgcctctgg
1501 gcttttagtg ccgcggggc ccggaggggc agcctttct cactgcagtc
10 agcgcggcaa
1561 ctataagagg cctataagag gcggtgccctc ccgcagtggc tgcttcagcc
cagcagccag
1621 gacagcgaac catgctgcct gcggcccgcc tccagactta ttagagccag
cctgggaact
15 1681 cgcattcactg ccctcaccgc tgtgtccagt cccaccgtcg cggacagcaa
ccacagtctg
1741 cagaaccgca gcacagaacc agcaaggcca ggcaggccat ggggctctgg
gcgcgtctgc
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ttgtctctgg
1921 gcaaagagcc aggcacgggc cttaccacgc tcccacgtcg tggggatcac
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2041 gaagtggggc acatcattgg catgcagaag cccagataca ccaggctcag
agaccattcc
2101 catttaatac gaccccgttt ctgctgagca acaggtccca acctcgctgt
30 ggtgggtct
2161 caggtgtccc ttaggtcttg aaccaaaaaa aaaaaaaaaa aaaaaaaaaa
accagatatt
2221 agctttgagg tgagggagtg gaattcctaa gttttcaag gtgggcaagg
ctgcagggtgg
35 2281 ggtttctct cgggggctga cttgaagaaa ggaagagcta aggttagccat
gcctttctg
2341 tccactcact agactctgga gctcaggggcc aggcaaggat agggtggtac
agcctgtatg
2401 gtttaggtgc aggtccccctc ccctggactg aaccctttagt catccggcca
40 gggcattctg
2461 gaacatagcc tcctccacga acctgttgac ggattccaag agtctgcagc
tggtgctcga
2521 gcccagtctg cagctgctga gccgcaagca gcccgcactg atccgacaga
accggggat

- 11 -

2581 cctgcacagc gtgagtgag ggctccagag cgctgtgcga gagtgcaaat
ggcaattccg
2641 aaaccgcccgc tggaactgcc ccactgctcc gggggccccac ctcttcggca
agatcgtcaa
5 2701 ccgaggtggg tgcccaggaa agcgacgctt ccgggattaa gggaaaagca
gggtcatctc
2761 cagggcatag gggggcgaag gcagggaaaga catcccaggg ttatatgtga
tcaaactgag
2821 aatcgctgg tgccggcagt taccgttagt cagcaccaga ttctttctag
10 ccttgcgttg
2881 tgagcatgat cttaacgtt gctggccact ggcccacaga aaggaaattc
cgatcgtgg
2941 ggcgtggcg acagctgttt ttccctagcc ttccctcaaag gtacctggga
agctgatctc
15 3001 tgagggctag ctagggttgt gtttcgcacc cagcaaagtt tgcactgcca
atactagtag
3061 cgatcttggc tatgcagatt tttttactt gggaaatctcc ctttggagct
gctctgttag
3121 ggctctggag tctcagtaaa gcttagagag gagggcattc catgttgc
20 acacatgact
3181 ccaaggatgt tggactgttag ggtaccaagt ttccaaaca gggtgcttag
ttggcccccac
3241 gccttctctc aactgatgcg gggtcgttcc acccacaggc tgccgagaaa
cagcgttcat
25 3301 cttcgcaatc acctccggcg gggtcacaca ttccgtggcg cgctcctgct
ccgaaggctc
3361 catcgagtcc tgcacctgcg actaccggcg ggcggccct gggggccccg
actggcactg
3421 ggggggctgc agtgacaaca tcgattttgg tcgcctcttt ggccgagagt
30 tcgtggactc
3481 cggggagaag gggcgggacc tacgcttcct catgaacctt cacaacaacg
aggcaggcg
3541 aacggtacgt cggtgtgtcc ggaaccaatg gcaggggaga tgtaagacag
gtgcacgggg
35 3601 acagaggcac agggaggggc ttcccggagag agtgggactc taggaggaa
gacagagaag
3661 aggtgggtggt tgagggcaaa gaggttcctg agctgatgac agaacagaag
agattagcag
3721 gctatcaaca cgtggatgt attgagatgg ctccatggca cactttgaa
40 agataaaaagt
3781 gacttgctgg cgtggagcag agtctggccg aatgtcccta tctcagcggg
ccatttgca
3841 cttcctctct cccgagctta gtcacacctg gaccttggct gaagtttcca
cagcatcgac

- 12 -

3901 gtgacccggg tgggggtgggg gtggggaaagt atgggtggtg gttcgtggga
tgttggcttt
3961 gaccttttct tccctcctcc cctcgtcccc tcctccccca gaccgtgttc
tctgagatgc
5 4021 gccaagagtg caaatgccac gggatgtccg gtcctgcac ggtgcgcacg
tgttggatgc
4081 ggctgcccac gctgcgcgct gtgggcgacg tgctgcgcga ccgccttcgac
ggcgccctccc
4141 gcgtccttta cggcaaccga ggcagcaacc gcgcctcgcg ggccggagctg
10 ctgcgcctgg
4201 agccccgaaga ccccgcgac aagcctccct cccctcacga cctcgtctac
ttcgagaaaat
4261 cggccaaactt ctgcacgtac agtggccgccc tggcacagc tggcacagct
ggacgagctt
15 4321 gcaacagctc gtctcccgcg ctggacggct gtgagctgct gtgctgtggc
cgaggccacc
4381 gcacgcgcac gcagcgcgtc acggagcgct gcaactgcac cttccactgg
tgctgcccacg
4441 tcagctgcgg caactgcacg cacacgcgcg ttctgcacga gtgtctatga
20 ggtggccgcg
4501 ctccgggaac gggAACGCTC tttccagtt ctcagacaca ctgcgtggc
ctgatgtttg
4561 cccaccctac cgcgtccagc cacagtccca gggttcatag cgatccatct
ctcccacctc
25 4621 ctacctgggg actcctgaaa ccacttgccc gagtcggctc gaaccctttt
gccatcctga
4681 gggccctgac ccagcctacc tccctccctc tttgagggag actccttttg
cactgcccc
4741 caatttggcc agagggtgag agaaagattc ttcttctggg gtgggggtgg
30 30 ggaggtcaac
4801 tcttgaagggt gttgcgggttc ctgatgtatt ttgcgtgtg acctctttgg
gtattatcac
4861 cttcccttgt ctctcggtc cctataggtc cttgaggttc tctaaccagc
acctctgggc
35 4921 ttcaaggct ttccctccc acctgttagct gaagagtttcc cgagttgaaa
gggcacggaa
4981 agctaagtgg gaaaggaggt tgctggaccc agcagcaaaa ccctacattc
tccttgcctc
5041 tgcctcgag ccattgaaca gctgtgaacc atgcctccct cagcctcctc
40 40 ccacccttc
5101 ctgtcctgcc tcctcatcac tgtgtaaata atttgcacccg aaatgtggcc
gcagagccac
5161 gcgttcgggtt atgtaaataa aactattat tgtgctgggt tccagcctgg
gttgcagaga

- 13 -

5221 ccaccctcac cccacccac tgctccctcg ttctgctcgc cagtccttt
 gttatccgac
 5281 cttttttctc ttttacccag cttctcatag ggcgccttgc ccaccggatc
 agtatttcct
 5 5341 tccactgttag ctattagtgg ctccctgccc ccaccaatgt agtatcttcc
 tctgaggaat
 5401 aaaatatcta ttttatcaa cgactctggt ctttgaatcc agaacacagc
 atggcttcca
 5461 acgtcccttcc cccctccaaat ggacttgctt ctcttctcat agccaaacaa
 10 aagagataga
 5521 gttgttgaag atctcttttc cagggcctga gcaaggaccc tgagatcctg
 acccttggat
 5581 gaccctaaat gagaccaact agggatc (SEQ ID NO:6)

Table 12: Human Wnt-2 Nucleotide Sequence

15 1 agcagagcgg acggggcgccg gggaggcgccg cagagcttcc gggctgcagg cgctcgctgc
 61 cgctgggaa ttgggctgtg ggcgaggcgccg tccgggctgg cctttatcgc tgcgtggcc
 121 catcggttga aactttatca gcgagtcgccc actcgctcga ggaccgagcg gggggcgcccc
 181 ggcgcggcgag gcggcgcccg tgacgaggcg ctcccggagc tgagcgccttc tgctctggcc
 241 acgcatggcg cccgcacacg gagtctgacc tgatgcagac gcaagggggt taatatgaac
 20 301 gcccctctcg gtggaatctg gctctggctc cctctgtct tgacctggct caccggcgg
 361 gtcaacttctt catgggtgta catgagagct acaggtggct cctccagggt gatgtgcgat
 421 aatgtgccag gcctgggtgag cagccagcg cagctgtgtc accgacatcc agatgtgtat
 481 cgtgccatta gccaggcggt ggccgagtgg acagcagaat gccagcacca gttccggccag
 541 caccgcttggaa attgcaacac cctggacagg gatcacagcc tttttggcag ggtcctactc
 25 601 cgaagtagtc gggaaatctgc ctttggat gccatctctt cagctggagt ttttggcc
 661 atcaccaggg cctgttagcca aggagaagta aaatctgtt cctgtgatcc aaagaagatg
 721 ggaagcgcca aggacagcaa aggatctttt gattgggggtg gctgcagtga taacattgac
 781 tatggatca aatttggcccg cgcatttgc gatgcaaagg aaaggaaagg aaaggatgac
 841 agagccctga tgaatcttca caacaacaga gctggcagga aggctgtaaa gcggttcttgc
 30 901 aaacaagagt gcaagtgcac cgggggtgagc ggctcatgtt ctctcaggac atgctggctg
 961 gccatggcccg acttcaggaa aacggggat tatctcttgc ggaagtacaa tggggccatc
 1021 caggtggtca tgaaccagga tggcacaggt ttcactgtgg ctaacgagag gttttaagaag
 1081 ccaacaaaaa atgacccctgt gtattttgag aattctccag actactgtat cagggaccga
 1141 gaggcaggct ccctgggtac agcaggccgt gtgtgcaacc tgacttcccg gggcatggac
 35 1201 agctgtgaag tcatgtgctg tgggagaggc tacgacaccc cccatgtcac cggatgacc
 1261 aagtgtgggt gtaagttcca ctgggtgtgc ggcgtgcgt gtcaggactg cctggaaagct
 1321 ctggatgtgc acacatgcaa ggcccccaag aacgctgact ggacaacccgc tacatgaccc
 1381 cagcaggcggt caccatccac ctcccttc acaaggactc cattggatct gcaagaacac
 1441 tggaccccttgg ttttttttgc gggggatat ttcctaaaggc atgtggcctt tatctcaacg
 40 1501 gaagccccctt ctccctccctt gggggccca ggtatgggggg ccacacgctg cacctaaagc
 1561 ctaccctattt ctatccatct cctgggttgc tgcagtcac tccctcctg gcgagttctc

- 14 -

1621 tttggaaata gcatgacagg ctgttcagcc gggagggtgg tgggccaga ccactgtctc
 1681 cacccacctt gacgtttctt ctttctagag cagttggcca agcagaaaaaa aaagtgtctc
 1741 aaaggagctt tctcaatgtc ttcccacaaa tggtcccaat taagaaattc catacttctc
 1801 tcagatggaa cagtaaagaa agcagaatca actgcccctg acttaacttt aacttttga
 5 1861 aagaccaaga cttttgtctg tacaagtggt tttacagcta ccacccttag ggtaatttgg
 1921 aattacctgg agaagaatgg ctttcaatac ccttttaagt taaaatgtg tatttttcaa
 1981 ggcattttt gccatattaa aatctgatgt aacaagggtgg ggacgtgtgt cctttggac
 2041 tatggtgtgt tttatcttg taagagcaaa agcctcagaa agggattgtt ttgcattact
 2101 gtccccttga tataaaaaat ctttagggaa tgagagttcc ttctcactta gaatctgaag
 10 2161 ggaataaaaa agaagatggaa tggctctggca atattctgtt actattgggt gaatatgtt
 2221 gaaaataatt tagtggatgg aatatcagaa gtatatctgtt acagatcaag aaaaaaaagga
 2281 agaataaaaat tcctatatca t (SEQ ID NO:7)

Table 13: Murine Wnt-3A Nucleotide Sequence

15 1 gaattcatgt cttacggtca aggcagaggg cccagcgcca ctgcagccgc
 gccacccccc
 61 agggccgggc cagcccaggc gtccgcgttc tcgggggtggc ctccccccgc
 tgcgcgctca
 121 agccggcgat ggctcctctc ggataacctct tagtgctctg cagcctgaag
 20 caggctctgg
 181 gcagctaccc gatctggtgg tccttggctg tgggacccca gtactccct
 ctgagcactc
 241 agcccattct ctgtgccagc atcccaggcc tggtaccgaa gcagctgcgc
 ttctgcagga
 25 301 actacgttggaa gatcatgccc agcgtggctg aggggtgtcaa agcgggcattc
 caggagtgcc
 361 agcaccagtt ccgaggccgg cgttggaaact gcaccaccgt cagcaacagc
 ctggccatct
 421 ttggccctgt tctggacaaa gccacccggg agtcagcctt tgtccatgcc
 30 atcgccctccg
 481 ctggagtagc ttgcgttcaggc acacgctctt gtgcagaggg atcagctgt
 atctgtgggt
 541 gcagcagccg cctccaggcc tccccaggcg agggctggaa gtggggccgc
 tgttagtgagg
 35 601 acattgaatt tggaggaatg gtctctcggtt agtttgcga tgccagggag
 aaccggccgg
 661 atgcccgttc tgccatgaac cgtcacaaca atgaggctgg gcccaggcc
 atcgccagtc
 721 acatgcaccc caagtgcacaa tgccacgggc tatctggcag ctgtgaagtg
 40 aagacctgtt
 781 ggtggtcgca gccggacttc cgcaccatcg gggatttcctt caaggacaag
 tatgacagtg
 841 cctcggagat ggtggtagag aaacaccgag agtctcgtgg ctgggtggag

- 15 -

accctgaggc
 901 cacgttacac gtacttcaag gtgccgacag aacgcgacct ggtctactac
 gaggcctcac
 961 ccaacttctg cgaacctaac cccgaaaccg gtccttcgg gacgcgtgac
 5 cgcacctgca
 1021 atgtgagctc gcatggcata gatgggtgcg acctgttgtg ctgcgggcgc
 gggcataacg
 1081 cgcgcactga gcgacggagg gagaaatgcc actgtgttt ccattggtgc
 tgctacgtca
 10 1141 gctgccagga gtgcacacgt gtctatgacg tgcacacctg caagtaggag
 agctcctaac
 1201 acgggagcag gttcattcc gaggggcaag gttctacct gggggcgggg
 ttcctacttg
 1261 gaggggtctc ttacttgggg actcggttct tacttgaggg cggagatcct
 15 acctgtgagg
 1321 gtctcataacc taaggacccg gttctgcct tcagcctggg ctcctatttg
 ggatctgggt
 1381 tccttttag gggagaagct cctgtctggg atacgggttt ctgcccggagg
 gtggggctcc
 20 1441 acttggggat ggaattccaa tttggccgg aagtcttacc tcaatggctt
 ggactcctct
 1501 cttgacccga cagggctcaa atggagacag gtaagctact ccctcaacta
 ggtggggttc
 1561 gtgcggatgg gtgggagggg agagattagg gtccctcctc ccagaggcac
 25 tgctctatct
 1621 agatacatga gagggtgctt cagggtgggc cctatttggg cttgaggatc
 ccgtgggggc
 1681 ggggcttcac cccgactggg tggactttt ggagaccccc ttccactggg
 gcaaggcttc
 30 1741 actgaagact catggatgg agtccacgg aaggaggagt tcctgagcga
 gcctgggctc
 1801 tgagcaggcc atccagctcc catctggccc cttccagtc ctggtgtaag
 gttcaacctg
 1861 caagcctcat ctgcgcagag caggatctcc tggcagaatg aggcatggag
 35 aagaactcag
 1921 gggtgataacc aagacctaac aaacccctgt cctgggtacc tctttaaag
 ctctgcaccc
 1981 cttcttcaag ggcttccta gtctccttgg cagagcttc ctgaggaaga
 tttgcagtcc
 40 2041 cccagagttc aagtgaacac ccatagaaca gaacagactc tattctgagt
 agagagggtt
 2101 ctcttaggaat ctctatgggg actgcttagga aggatcctgg gcatgacagc
 ctcgtatgat
 2161 agcctgcata cgctctgaca cttataactc agatctcccg gaaacccag

- 16 -

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ctcatccggt
  2221 ccgtgatgtc catgccccaa atgcctcaga gatgttgctt cactttgagt
tgtatgaact
  2281 tcggagacat ggggacacag tcaagccgca gagccagggt tgtttcagga
5 cccatctgat
  2341 tccccagagc ctgctgttga ggcaatggtc accagatccg ttggccacca
ccctgtcccg
  2401 agcttctcta gtgtctgtct ggcttggaa tgagggtgcta catacagccc
atctgccaca
10  2461 agagcttcctt gattggtacc actgtgaacc gtccctccccc ctccagacag
gggaggggat
  2521 gtggccatac aggagtgtgc ccggagagcg cgaaagagg aagagaggct
gcacacgcgt
  2581 ggtgactgac tgtcttctgc ctggaaacttt gcgttcgcgc ttgttaacttt
15 attttcaatg
  2641 ctgctatatac caccaccac tggatttaga caaaagtgtat tttttttttt
ttttttctt
  2701 ttctttctat gaaagaattt attttagttt atagttatgtt tgtttcaaatt
aatggggaaa
20  2761 gtaaaaaagag agaaaaaaaaaaa aaaaaaaaaaaa aaaaaaaaaaaa aaaa
(SEQ ID NO:8)

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Table 14: Human Wnt-3a nucleotide sequence

```

tgtaagtgcc acgggctgtc gggcagctgc gaggtgaaga catgtgggtg
gtcgcaaccc gacttccgcg ccatcggtga cttcctcaag gacaagtacg
25 acagcgccctc ggagatggtg gtggagaagc accgggagtc ccgcggctgg
gtggagaccc tgccggccgcg ctacacctac ttcaagggtgc ccacggagcg
cgacacctgtc tactacgagg cttcgcccaa cttctgcgag cccaaacctg
agacgggctc cttcggcactg cgccgaccgca cctgcaacgt cagctcgac
ggcatcgacg gctgcaactt gctgtgtgc ggccgcggcc acaacgcgcg
30 ageggagcgg cgccggaga agtgcggctg cgtgtttcac tggtgctgt
(SEQ ID NO:9)

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Human nucleic acid sequences which encode Wnt-4, Wnt-7a, and Wnt-7b are shown Tables 15, 16, and 17, respectively.

35 Human and mouse Wnt polypeptides function similarly in transformation assays. Accordingly, human or mouse Wnt polypeptides or nucleic acids are administered to mammals to therapeutically stimulate

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tubulogenesis. The amino acid and nucleotide sequences of Wnt polypeptides are known in the art, e.g., human Wnt-1 (GENBANK® X03072), human Wnt-2 (GENBANK® X07876), human Wnt-4 (GENBANK® AAB30677), human Wnt-7a (GENBANK® 5 000755), mouse Wnt-1 (GENBANK® P04426), mouse Wnt-2 (GENBANK® P21552), mouse Wnt-3a (GENBANK® P27467), mouse Wnt-4 (GENBANK® P22724 and M89787), mouse Wnt-7a (GENBANK® M89802), and mouse Wnt-7a (GENBANK® M89801).

Kidney tubulogenesis is a multi-step process with 10 a hierarchy of signaling systems. A permissive signal from the ureter to the mesenchyme triggers survival and tubulogenesis in the mesenchyme, signals from the mesenchyme to the ureter are required for proliferation and branching morphogenesis of the ureter. Other 15 signaling systems within the ureter are required for local adhesion and proliferation, changes which may mediate branching morphogenesis, and within the mesenchyme, for tubulogenesis as evidenced by the role of Wnt-4.

20 The data described herein indicate that Wnt-4 is sufficient to trigger tubulogenesis in isolated metanephric mesenchyme, whereas Wnt-11 which is expressed in the tip of the growing ureter is not. Wnt-4 signaling depends on cell contact and sulphated glycosaminoglycans. 25 Wnt-4 is required for triggering tubulogenesis but not for later developmental events. The Wnt-4 signal can be replaced by other members of the Wnt gene family including Wnt-1, Wnt-3a, Wnt-7a and Wnt-7b. Further, dorsal spinal cord, which has been thought to mimic 30 ureteric signaling in tubule induction, induces Wnt-4 mutant as well as wild-type mesenchyme suggesting that spinal cord derived signal(s) likely act by mimicking the normal mesenchymal action of Wnt-4. These results indicate that Wnt-4 is a key auto-regulator of the 35 mesenchymal to epithelial transformation that leads to

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tubulogenesis and nephrogenesis.

Therapeutic administration of a Wnt polypeptide or agonist

Wnt polypeptides or agonists are useful to treat
5 kidney disorders such as chronic renal insufficiency,
end-stage chronic renal failure, glomerulonephritis,
glomerulosclerosis, interstitial nephritis,
pyelonephritis, kidney failure due to viral disease,
kidney failure after transplantation.

10 Wnt polypeptides are at least about 10 amino acids, usually about 20 contiguous amino acids, preferably at least 40 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least about 60 to 80 contiguous amino acids
15 in length and have the biological activity of triggering tubulogenesis. For example, a Wnt polypeptide is at least 50% of the length of the corresponding naturally-occurring Wnt polypeptide and has the amino acid sequences of the amino-terminal half of the naturally-
20 occurring polypeptide. Such peptides are generated by methods known to those skilled in the art, including proteolytic cleavage of the protein, *de novo* synthesis of the fragment, or genetic engineering, e.g., cloning and expression of a fragment of Wnt-encoding cDNA.
25 Therapeutic compositions are administered in a pharmaceutically acceptable carrier (e.g., physiological saline). Carriers are selected on the basis of mode and route of administration and standard pharmaceutical practice. A therapeutically effective amount of a
30 composition (e.g., Wnt polypeptide or agonist) is an amount which is capable of producing a medically desirable result, e.g., tubulogenesis, in a treated animal. As is well known in the medical arts, dosage for any one animal depends on many factors, including the
35 animal's size, body surface area, age, the particular

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compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently (e.g., other Wnt polypeptides) is 0.1 to 100 mg/kg body weight. Administration is 5 generally be parenterally, e.g., intravenously, subcutaneously, intramuscularly, or intraperitoneally. The compositions of the invention can be administered locally i.e., at the site of organ damage or systemically. For example, the route of delivery is by 10 intravenous infusion, localized injection or implants. The polypeptides or agonists may be formulated so as to have a continual presence in the tissue during the course of treatment, e.g., by being covalently attached to a polymer such as polyethylene glycol (PEG). Such 15 continuous release formulations are administered at weekly intervals or at multiples of weekly intervals. Examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the polypeptide or agonist, which matrices are 20 in the form of shaped films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer et al., 1981, J. Biomed. Mater. Res., 15: 167-277 and Langer, 1982, Chem. Tech., 12: 98-105 or 25 poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., 1983, Biopolymers, 22: 547-556), non-degradable ethylene-vinyl acetate (Langer et al., *supra*), degradable lactic 30 acid-glycolic acid copolymers, polylactate polyglycolate (PLGA), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988). While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid provide release of molecules for over 100 days, certain hydrogels release 35 proteins for shorter time periods.

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Sustained-release Wnt compositions also include liposomally entrapped Wnt polypeptides or agonists. Liposomes containing Wnt compositions are prepared by methods known in the art, e.g., Epstein et al., 1985, 5 Proc. Natl. Acad. Sci. USA, 82: 3688-3692; Hwang et al., 1980, Proc. Natl. Acad. Sci. USA, 77: 4030-4034; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. The compositions may also be administered directly to a tissue site, e.g., by biolistic delivery to an internal 10 or external target site or by catheter into a body lumen. Therapeutic compositions are administered by retrograde perfusion of kidney via the ureter or other urine collecting lumens, e.g., using a catheter or perfusion apparatus, such as that described in U.S. Pat. No. 15 5,871,464.

Analogs, homologs, or mimetics of the above peptides may also be used to induce and promote kidney tubule formation in a post-natal mammal. Analogs can differ from the naturally-occurring Wnt polypeptides by 20 amino acid sequence, or by modifications which do not affect the sequence, or both. Modifications (which do not normally alter primary sequence) include *in vivo* or *in vitro* chemical derivitization of polypeptides, e.g., acetylation or carboxylation. Also included are 25 modifications of glycosylation, e.g., those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps, e.g., by exposing the polypeptide to enzymes which affect glycosylation, e.g., mammalian 30 glycosylating or deglycosylating enzymes. To improve the solubility and therapeutic half-life of Wnt polypeptides, Wnt-Ig fusion proteins are produced. Methods of making Ig fusion proteins is well known in the art (e.g., as described in Current Protocols of Immunology, 1994, 35 Coligan et al., eds., John Wiley & Sons, Inc., p.

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10.19.1-10.19.11).

To render the therapeutic peptides less susceptible to cleavage by peptidases, the peptide bonds of a peptide may be replaced with an alternative type of 5 covalent bond (a "peptide mimetic"). Where proteolytic degradation of the peptides following injection into the subject is a problem, replacement of a particularly sensitive peptide bond with a noncleavable peptide mimetic renders the resulting peptide more stable, and 10 thus more useful as a therapeutic. Such mimetics, and methods of incorporating them into polypeptides, are well known in the art. Similarly, the replacement of an L-amino acid residue with a D-amino acid is a standard way of rendering the polypeptide less sensitive to 15 proteolysis. Also useful are amino-terminal blocking groups such as t-butyloxycarbonyl, acetyl, theyl, succinyl, methoxysuccinyl, suberyl, adipyl, azelayl, dansyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, methoxyazelayl, methoxyadipyl, methoxysuberyl, and 2,4,- 20 dinitrophenyl. Peptides may be administered to a subject intravenously in a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are biologically compatible vehicles which are suitable for administration to an animal: e.g., physiological saline.

25 Wnt polypeptides are generally administered *in vivo* to allow regeneration of kidney tissue in the context of the autologous organ. However, kidney tissue or dissociated cells (derived from kidney tissue or embryonic tissue) may be treated outside the body (i.e., 30 *ex vivo*) and then transplanted back into the body from which it was derived or into a different mammal. In the case of *ex vivo* therapy, a damaged or diseased kidney is removed from an individual, treated with a Wnt polypeptide (or DNA encoding a Wnt polypeptide) and then 35 transplanted into the same individual or a different

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individual.

Therapeutic administration of DNA encoding a Wnt polypeptide or agonist

Gene therapy for regeneration of kidney tissue is 5 carried out by directly administering the claimed DNA to a mammal or by transfecting kidney cells, e.g., renal mesenchymal cells or endothelial cells, with Wnt-encoding DNA *in vivo* or *ex vivo*. Gene transfer into kidney tissue is carried out using known methods, e.g., bathing the 10 tissue or cells in a solution containing Wnt-encoding DNA. Alternatively, kidney tissue is perfused *in vivo* or explanted kidney tissue is perfused *ex vivo*, using a perfusion apparatus, such as that described in U.S. Pat. No. 5,871,464. After the cells are contacted with DNA, 15 the cells or organ is transplanted into a recipient (or returned to the host from which it was removed). If the cells in suspension, the cells are infused into the mammal to be treated.

To express a Wnt polypeptide in a kidney cell, a 20 Wnt-encoding DNA is introduced into a target cell, e.g., a mesenchymal or epithelial kidney cell, of the mammal by standard vectors and/or gene delivery systems. For example, expression of exogenous Wnt DNA in an epithelial cell induces production and secretion of a Wnt 25 polypeptide, which in turn, leads to tubulogenesis and kidney regeneration. Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, adenovirus, and adeno-associated virus, 30 among others. A therapeutically effective amount is an amount of the nucleic acid of the invention which is capable of producing a medically desirable result in a treated animal, e.g., tubulogenesis.

DNA or transfected cells may be administered in a 35 pharmaceutically acceptable carrier. Pharmaceutically

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acceptable carriers are biologically compatible vehicles which are suitable for administration to a mammal, e.g., physiological saline. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages will vary, but a preferred dosage for intravenous administration of DNA is from approximately 10^6 to 10^{22} copies of the DNA molecule. The compositions of the invention may be administered locally or systemically. As with other therapeutic compositions such as peptides, administration of a nucleic acid composition is generally be parenterally, e.g., intravenously. DNA is also administered by retrograde perfusion of kidney tissue using, e.g., a catheter. DNA may also be administered directly to the target site, e.g., by biolistic delivery to a kidney tissue or by an implantable device.

Methods of delivering nucleic acids to kidney tissue are known in the art, e.g., those described by Sukhatme et al. in U.S. Pat. No. 5,869,230. Nucleic acids are expressed under the control of tissue-specific, e.g., kidney-specific, promoters such as the Pax-2 promoter, the cRET promoter, and the Hox b7 promoter. Promoter constructs for inducible and constitutive expression of heterologous sequences are well known in the art and commercially-available. For example, nucleic acids are expressed under the control of the cytomegalovirus (CMV) β -actin promoter for general constitutive expression.

Method of screening for compounds which increase Wnt expression

A screening assay to identify compounds which are capable of inducing or increasing Wnt polypeptide

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expression in kidney tissue of a post-natal mammal (i.e., non-embryonic cells) is carried out as follows. For example, a sample of kidney cells, e.g., cultured mesenchymal or epithelial cells, is incubated in the 5 presence of a candidate compound. A sample of control cells is incubated in the absence of the compound. Each sample of cells is evaluated for the expression of a Wnt polypeptide, e.g., Wnt-4. To test for presence of the Wnt gene product, each sample of cells can be incubated with 10 a Wnt-specific antibody and the cells evaluated for binding of the antibody by methods well known in the art, e.g., immunofluorescent staining. The amount of antibody binding correlates with the level of expression of the Wnt polypeptide. Wnt expression is also measured at the 15 level of gene transcription. For example, Wnt transcripts can be measured by Northern blotting techniques using Wnt-specific DNA probes or by PCR using Wnt-specific DNA primers. A increase in the amount of 20 Wnt gene expression in cells contacted with a candidate compound compared to the amount in untreated cells indicates that the candidate compound is capable of inducing or increasing the expression of a Wnt polypeptide in kidney cells (and inducing tubulogenesis). The compound is tested in tissue or organ culture systems 25 as described below to determine whether the compound triggers tubulogenesis.

Mouse model of renal development

Mouse renal development is characterized by the continuous interaction of epithelial and mesenchymal 30 compartments both of which are derived from the intermediate mesenchyme. These compartments are the nephric duct and its derivative, the ureter, and the nephrogenic mesenchyme which lies adjacent to these ducts. As a consequence of these interactions, three 35 embryonic kidneys are laid down from anterior to

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posterior in time and space. While the initial organ, the pronephros is only a very transient structure established at 8-8.5 days post coitum (d.p.c.), the mesonephros extends by posterior elongation of the 5 nephric duct and subsequent tubule induction in the adjacent mesonephrogenic mesenchyme between 9 and 11 d p.c. Although forming elaborate tubules, the mesonephros of the male never becomes a functional organ but contributes to the ductal network of the rete testis.

10 Metanephric development is initiated when a bud emerges from the nephric duct at the level of the hind limbs around 10.5 d.p.c. The ureteric duct subsequently invades the metanephric blastema which lies at the posterior end of the intermediate mesoderm.

15 In a process repeated many times, mesenchymal cells condense around the tip of the ureter, i.e., bud, aggregate, epithelialize and undergo morphogenetic movements. Cellular differentiation occurs to form a major part of the nephron, the functional unit of the 20 vertebrate kidney. The ureter continues to grow and to branch forming the collecting duct system of the mature organ. 7-10 days post partum, nephron formation ceases as the mesenchymal stem cells in the periphery of the kidney are exhausted.

25 The role of Wnt-11, Wnt-4 and other Wnt family members in tubule induction was studied. Wnt-4, but not Wnt-11 was found to be able to induce tubule formation, suggesting that spinal cord mediated tubulogenesis reflects the normal mesenchymal function of Wnt-4 rather 30 than that of a ureteric bud derived signal.

The following reagents and procedures were used to evaluate Wnt signalling in the developing kidney.

Mice

Wnt-4 heterozygotes were derived and genotyped 35 using known methods, e.g., that described by Stark et

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al., 1994, *Nature* 372:679-683. Embryos for kidney dissections were derived from matings of Swiss Webster (SW) wild-type animals or Wnt-4 heterozygotes. For timed pregnancies, plugs were checked in the morning after 5 mating, noon was taken as 0.5 d.p.c.

Cell lines

Cell lines which stably express various Wnt genes or LacZ were prepared using standard methods, e.g., that described by Pear et al., 1993, *Proc. Natl. Acad. Sci.* 10 USA 90: 8392-8396. For Wnt polypeptide expression, full-length cDNAs encoding Wnt-1 (van Ooyen and Nusse, 1984, *Cell* 39: 233-240), Wnt-3a (Roelink and Nusse, 1991, *Genes Dev.* 5: 381-388), Wnt-4, Wnt-5a, Wnt-7a, Wnt-7b (Gavin et al., 1990, *Genes Dev.* 4: 2319-2332), Wnt-11 (Kispert et al., 1996, *Development* 122:3627-3637) and lacZ were cloned into an expression vector, e.g., the retroviral expression vector pLNCX which confers expression of foreign genes under the control of the CMV promotor. Bosc23 packaging cells were transfected with recombinant 20 DNA constructs. Viral supernatants were collected 48-72 h later and used to infect standard NIH3T3 cells. After 10 d of selection in G418, pools of cells were used for recombination experiments. 50,000 cells were plated in 50 μ l of medium on polycarbonate filter and grown for 25 18-24 h at 37°C in 5% CO₂.

Organ culture techniques

Metanephric kidneys from SW or Wnt-4 intercrosses were dissected in phosphate buffered saline (PBS). To generate a preparation of dissociated kidney cells from 30 embryonic or mature tissue, the tissue is dissected and enzymatically digested. For example, metanephric mesenchyme was dissected manually from the ureter (bud stage [10.75 d.p.c.] to early T stage [11.5 d.p.c.]), following a 2 min. incubation in 3% pancreatin/trypsin 35 (GibcoBRL) in Tyrode's solution. In recombination

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experiments with wild-type mesenchymes, samples were pooled before being distributed to individual experiments. In experiments with Wnt-4 mutant embryos, metanephric mesenchyme from each kidney of the embryo was 5 kept separate. The remainder of an embryo was used for genotyping by Southern analysis. In recombination experiments with dorsal spinal cord, metanephric mesenchyme from two kidneys was surrounded by two dissected pieces of dorsal spinal cord from the same 10 embryo on a 1 μm polycarbonate filter (Costar). For direct recombination experiments with Wnt-expressing cells, two mesenchymes were placed on top of modified NIH3T3 cells. For transfilter experiments, 50,000 cells in 50 μl medium were seeded on a 1 μm filter 18-24 h 15 prior to the recombination. Cells were then covered with a 1 μm filter and two mesenchymes placed on this filter. Filters (4-6 mm in size) were supported by stainless steel grids on the surface of the culture medium (Dulbecco's modified Eagle's medium supplemented with 10% 20 fetal calf serum, 2 mM glutamine, 1 \times penicillin/streptomycin). Medium was changed every 2 d. For studies of glycosaminoglycan dependence of tubule induction, the medium was supplemented with 30 mM NaClO₃ after 0 h, 24 h and 48 h, respectively. In experiments 25 concerning pore size dependence of induction, the pore size of the upper filter in the transfilter set-up was varied from 0.05 μm , 0.1 μm , 0.4 μm , 0.8 μm to 1 μm . For marker experiments, at least 6 specimens were processed.

For *in situ* hybridization analysis, filters were 30 submerged in cold methanol for 10 seconds and then fixed in 4% paraformaldehyde in PBS overnight prior to stepwise transfer into methanol and storage at -20°C. For histological analysis, filters were fixed in Bouin's solution and stored in 70% ethanol at 4°C.

35 *In situ* hybridization analysis

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In situ hybridization analysis on whole mount cultures were performed using standard methods. Full-length cDNAs for WT-1 (Pritchard-Jones et al., 1990, Nature 346:194-197), Pax-2 (Dressler et al., 1990, 5 Development 109:787-795), Pax-8 (Plachov et al., 1990, Development 110:643-651), Wnt-4 (Gavin et al., 1990, Genes Dev. 4:2319-2332) and E-cadherin (Ringwald et al., 1987, EMBO J. 6:3647-3653) were labeled with Digoxigenin for whole mount detection.

10 Histological analysis and documentation

Samples were dehydrated, embedded in wax and sectioned at 5 μ m. Sections were dewaxed, rehydrated and stained with haematoxylin and eosin. Brightfield images of cultures and marker stainings were taken with a 15 binocular on Kodak 64T slide film. Histological sections were photographed on the same film on a Leitz Axiophot. Slides were scanned and documented in Adobe Photoshop 4.0.

20 Spinal cord mimics a mesenchymal signal for tubule induction

The identification of Wnt-4 as a mesenchymal signal essential for tubule formation provides a strategy for evaluating the role of spinal cord explants as heterologous inducers of kidney tubulogenesis. If the 25 spinal cord mimics a ureteric signal upstream of Wnt-4, this signal would not rescue the mesenchymal requirement for Wnt-4 in tubulogenesis. To test this possibility, isolated metanephric mesenchyme from individual embryos derived from intercrosses between mice heterozygous for a 30 likely null allele of Wnt-4 were cultured on a polycarbonate filter in direct contact with dorsal spinal cord from the same embryo. In the absence of spinal cord, all mesenchyme cultures rapidly degenerated as expected. Surprisingly, when cultured in the presence of 35 spinal cord, mesenchyme from Wnt-4 mutant embryos

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developed as well as that of wild-type or heterozygous siblings (Table 1).

Table 1: Induction of tubulogenesis in Wnt-4/Wnt-4 mutant metanephric mesenchyme by dorsal spinal cord

5	Exp #	# Recombinants	#induced/#total		
			+/+	Wnt-4/+	Wnt-4/Wnt-4
	1	8	2/2	5/5	1/1
	2	7	1/1	3/3	3/3
	3	7	3/3	3/3	1/1
10	4	5	1/1	3/3	1/1
	5	9	3/3	4/4	2/2
	6	11	7/7	4/4	-
	7	11	3/3	4/4	4/4
	Total	58	20/20	26/26	12/12

15 Isolated metanephric mesenchyme was recombined with dorsal spinal cord from the same embryo on a nucleopore filter. Induction was monitored by bright field microscopy. Embryos of a total of seven litters were analyzed.

20 Induction of tubulogenesis in wild-type and Wnt-4 mutant metanephric mesenchyme by dorsal spinal cord was analyzed as follows. Isolated metanephric mesenchyme and dorsal spinal cord from the same 11.5 d embryo were recombined on a nucleopore filter. After 48 h and 96 h, 25 cultures were monitored as whole mounts using bright field microscopy; after 144 h, they were analyzed as histological sections. Induction of tubulogenesis in wild-type and Wnt-4/Wnt-4 mutant metanephric mesenchyme were indistinguishable.

30 After 48 h, induction was visible as bright round zones of condensing mesenchyme. After 96 h, the zones of

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condensing mesenchyme had undergone epithelialization to form complex tubules. At 144 h, epithelial tubular structures and glomeruli indicated that full differentiation of induced tubules occurred in all 5 recombinants.

The induction of tubulogenesis in Wnt-4 mutant mesenchyme indicates that spinal cord signaling acts by either mimicking the action of Wnt-4 itself, or a factor downstream of Wnt-4. Further, although Wnt-4 is 10 expressed in the spinal cord, the observation that spinal cord from Wnt-4 mutants is capable of induction indicates that Wnt-4 expression in the spinal cord is not essential for this process, suggesting the involvement of other Wnts expressed in this tissue.

15 Wnt polypeptides which are sufficient to trigger tubulogenesis

In order to investigate whether Wnt-4 is sufficient for tubulogenesis, and if this property is shared by other Wnts normally expressed in the spinal 20 cord, NIH3T3 cell lines which stably express various Wnt genes were established. Direct recombinations were performed between Wnt-expressing cells and isolated wild-type metanephric mesenchyme.

Isolated metanephric mesenchyme from 2-3 11.5 d 25 kidneys was placed on top of NIH3T3 cells expressing various Wnt genes. As a control, mesenchymes were placed on NIH3T3 cells expressing LacZ and were placed onto a filter without an underlying cell layer. Induction was scored after 6 d using the morphological appearance of 30 the culture (as documented by brightfield microscopy), and histological analysis of selected samples. For each cell type 2-3 independent experiments were performed.

Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing various Wnt genes 35 was evaluated as follows. Brightfield microscopy (24 h,

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88 h) and histological analysis (144 h) of direct recombinations between NIH3T3 cells expressing Wnt genes and isolated metanephric mesenchyme. After 24 h, bright zones indicating induction were visible in recombinants 5 between wild-type mesenchyme and Wnt-1, Wnt-3a, Wnt-4, Wnt-7a and Wnt-7b expressing cells. These condensing mesenchymal cells had epithelialized and formed tubular structures after 88 h. After 144 h highly elaborate tubular structures were apparent. In contrast, cells 10 expressing Wnt-5a, Wnt-11, or as a control lacZ, respectively, did not support survival and differentiation of metanephric mesenchyme.

Co-cultures with Wnt-1, Wnt-3a, Wnt-4, Wnt-7a and Wnt-7b expressing cells developed on schedule with those 15 induced by spinal cord and formed complex epithelial tubules with differentiated glomeruli at 144 h (Table 2). In contrast, cells expressing Wnt-5a, Wnt-11 or a lacZ control did not support survival and differentiation of metanephric mesenchyme (Table 2).

20 Table 2: Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing various Wnt genes

	Cell line	#induced/#total
25	Wnt-1	16/16
	Wnt-3a	14/14
	Wnt-4	14/14
	Wnt-5a	0/12
	Wnt-7a	12/12
	Wnt-7b	11/12
30	Wnt-11	0/12
	LacZ	1/14
	mesenchyme	1/12
Wnt mRNA expression was comparable amongst the		

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various lines. These data indicate that a subset of Wnt genes, which includes Wnt-4 and not Wnt-11, induces tubule formation. As all of these are expressed in the spinal cord at the time of assay, it is likely that these 5 signals account for the robust inducing activity of the spinal cord. However, of these Wnt-4 is the only member which is actually expressed in and which is also required for mesenchymal aggregation.

Wnt-4 triggers the complete program of tubular 10 differentiation

In order to investigate whether Wnt-4 is sufficient to induce fully developed tubules in isolated metanephric mesenchyme, the induction properties of NIH3T3 cells expressing Wnt-4 were analyzed more 15 carefully by assessing the differentiation state of the mesenchyme by histological and molecular criteria.

Histological analysis of tubule induction in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 was evaluated as follows. NIH3T3 cells 20 expressing Wnt-4 were recombined with isolated metanephric mesenchyme directly and in a transfilter set-up. Cultures were analyzed by sectioning and histological staining after 24 h, 48 h, 96 h and 192 h of culture. Tubule induction in transfilter assays appeared 25 slightly delayed compared to direct recombinations. After 48 h, zones of condensed and aggregated mesenchyme were detected, and after 96 h, epithelial tubules were apparent. After 8 d in culture, fully differentiated tubular structures including glomeruli were detected.

30 Tubule induction by spinal cord was demonstrated in the art-recognized system in which cells are cultured with polycarbonate filters of a certain pore size (e.g., the method described by Grobstein, 1956, Science 118:52-55). Wnt-4-expressing cells were seeded on one filter; 35 these cells were separated from isolated mesenchyme by

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another filter of 1 μ m pore size. Induction took place transfilter, though with a delay when compared with direct recombinants.

Transfilter cultures appeared less compact and 5 flatter. Zones of condensed mesenchyme formed after 24 h, and aggregating mesenchyme and simple epithelial bodies appeared after 48 h. Epithelial tubules were seen after 96 h, and glomeruli were detected by 8 days.

To verify that these morphological features 10 reflected an underlying differentiation of the mesenchyme in response to Wnt-4, the temporal and spatial expression of a number of molecular markers was examined. Marker analysis of tubule induction in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 were analyzed 15 as follows. NIH3T3 cells expressing Wnt-4 were recombined with isolated metanephric mesenchyme in a transfilter set-up and scored for marker expression by *in situ* analysis after 24 h, 48 h, 96 h and 192 h of culture, respectively. Expression of WT-1, Pax-2, Pax-8, 20 Wnt-4 and E-cadherin, respectively, were in accordance with expression data from *in vivo* and *in vitro* studies of tubular differentiation.

WT-1 was broadly expressed after 1 d refining to small intensely labeled foci by 8 days of culture. This 25 expression profile parallels the expression of this gene during metanephric development which is first expressed in condensing mesenchyme, then in simple epithelial bodies before it is restricted to podocytes in the glomeruli. In the recombinants, WT-1 expression was 30 detected in glomeruli after 8 d in agreement with the histological analysis. Like WT-1, Pax-2 is also broadly expressed after 1 d, but becomes restricted to epithelial bodies and is lost after 4 d reflecting initial expression in condensing metanephric mesenchyme, 35 continuing expression in simple epithelial bodies and

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subsequent down-regulation as glomeruli start to differentiate. Wnt-4 is expressed in aggregating mesenchyme, in the epithelial bodies which they generate and is subsequently down-regulated as these mature into 5 S-shaped bodies. Pax-8, a paired-box transcription factor, has a similar early expression to Wnt-4 which has been shown to depend on Wnt-4 activity. In cultures, Wnt-4 was transiently expressed between 24 h and 96 h, peaking at 48 h. Pax-8 expression extended longer in S-10 shaped bodies. E-cadherin, which is expressed in the proximal tubules *in vivo*, was present after 24 h and was maintained, consistent with the differentiation of epithelial tubules along the proximal distal axis.

These data indicate that tubulogenesis in isolated 15 metanephric mesenchyme induced by Wnt-4 follows a similar progression to that observed in the metanephric kidney *in vivo*. At the stage at which the metanephric mesenchyme (T-stage of the ureter) was isolated, initial ureteric signaling had occurred, as evidenced by the condensation 20 of mesenchyme around the tip of the ureteric bud. However, this alone is insufficient to support mesenchymal survival and tubulogenesis. In contrast, Wnt-4 expressing cells were sufficient to support these 25 processes. In order to exclude that Wnt-4 only maintains Wnt-4 expression in the isolated mesenchyme, mesenchyme derived from 10.75 d.p.c. embryos was also analyzed. At this stage, the ureter bud had just emerged and the metanephric mesenchyme can first be identified. Wnt-4 expressing cells triggered the complete differentiation 30 program as judged by brightfield observation (12 out of 12 cases) and by molecular criteria (Pax-8 induction in 8 out of 8 cases after 4 d of culture).

Wnt-4 signaling requires cell contact

Tubule induction in isolated metanephric 35 mesenchyme was analyzed with respect to filter pore size.

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Experiments using the spinal cord as a heterologous inducer suggest a requirement for cell-cell contact as pore sizes below 0.1 μm , which prevent the extension of cytoplasmic processes, block induction.

5 Pore size dependence of tubule induction by Wnt-4 expressing cells was tested as follows. NIH3T3 cells expressing Wnt-4 were recombined with isolated metanephric mesenchyme in a transfilter set-up with various pore sizes of the nucleopore filter. Induction 10 was scored after 4 d by Pax-8 expression in whole mount *in situ* analysis. Pore sizes of 0.1 μm and bigger supported full induction of metanephric mesenchyme, whereas 0.05 μm pore size reduced or abolished induction (Table 3).

15 Table 3: Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 in transfilter assays with increasing pore size

	<u>Pore size</u>	<u># induced/# total</u>
20	0.05 μm	3*/13
	0.1 μm	14/16
	0.4 μm	14/14
	0.8 μm	6/6
	1 μm	3/3

* In each of the specimen scored as induced, only 1-4 25 spots of Pax-8 expression were seen in contrast to 15-30 with all the other pore sizes.

Supernatants from Wnt-4 expressing cells alone did not induce tubulogenesis, suggesting that cell contact is required. Wnt-4 may act as an insoluble cell bound 30 factor or it may associate with the extracellular matrix (ECM). It is unlikely that Wnt-4 mediated induction occurs through a secondary, soluble factor.

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Wnt-4 signaling requires sulphated glycosaminoglycans

Experiments were carried out to determine whether Wnt signaling for tubule induction depends on sulfated glycosaminoglycans (GAG)s which might act as cofactors 5 for binding the Wnt protein on the responsive cell. Accordingly, studies were undertaken to see evaluate whether the presence of 30 mM NaClO₃ (a competitive inhibitor of sulfation of GAGs) affects tubule induction. NIH3T3 cells expressing Wnt-4 were recombined with 10 isolated metanephric mesenchyme in a transfilter set-up with addition of 30 mM NaClO₃ in the medium. NaClO₃ was added to cultures at the start of transfilter culture, or 24 and 48 h later. As a control, chlorate was omitted completely. Induction was scored after 4 d by Pax-8 15 expression using whole mount *in situ* hybridization analysis. Addition of 30 mM NaClO₃ after 24 h or 48 h of culture did not affect tubule induction compared to untreated controls, whereas administration of 30 mM NaClO₃ at the beginning of the culture abrogated tubule 20 induction completely (Table 4).

Table 4: Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 in presence of 30 mM NaClO₃,

30 mM NaClO ₃ added 25 after h in culture	#induced/#total
0 h	0/19
24 h	12/19
48 h	14/17
-	12/15

30 When chlorate was added at 0 h, mesenchyme degenerated and Pax-8 expression was consequently negative. However, addition of chlorate after 24 h did

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not influence Pax-8 expression. Hence, GAGs are not involved in tubule maturation and differentiation. Tubule induction does, however, depend on sulfated GAGs in the first 24 h, the period essential for complete 5 induction by the spinal cord.

The chlorate inhibition experiments define a critical period of 24 h for induction. Further differentiation, i.e. aggregation and epithelialization of mesenchymal cells is only initiated when a certain 10 number of cells (a small community) has received the Wnt-4 signal. At this time, mesenchymal development is independent of ureteric signaling.

Chlorate acts as a competitive inhibitor of sulphotransferases and inhibits the sulphation of 15 glycosaminoglycans. The inhibition studies point to a critical role of these ECM compounds in tubulogenesis. Numerous studies have shown that branching morphogenesis of the ureter as well as branching of other epithelia requires an intact ECM. Since presence of chlorate after 20 24 h does not influence tubulogenesis, GAGs do not seem to be involved in tubule maturation and differentiation. Tubule induction does, however, depend on sulfated GAGs in the first 24 h, the period essential for complete 25 induction by the spinal cord. GAGs may act as co-receptors, facilitating presentation or increasing the local concentration of the ligand.

Wnt-4 signaling as a trigger for tubulogenesis

In order to test whether Wnt-4 expressing cells can rescue a Wnt-4 mutant mesenchyme, direct 30 recombination experiments were carried out in culture. Induction of tubulogenesis in wild-type and Wnt-4 mutant metanephric mesenchyme by NIH3T3 cells stably expressing Wnt-4 was evaluated as follows. Isolated metanephric mesenchyme was placed on top of NIH3T3 cells expressing 35 Wnt-4 which were supported by a nucleopore filter. After

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48 h and 96 h, cultures were monitored as whole mounts using bright field microscopy; after 144 h, the cultures were monitored as histological sections. Induction of tubulogenesis in wild-type and Wnt-4/Wnt-4 mutant 5 metanephric mesenchyme by Wnt-4 expressing cells were indistinguishable. Wnt-4-expressing cells were equally efficient at inducing tubule formation in wild type or Wnt-4 mutant metanephric mesenchyme (Table 5).

Brightfield microscopy and histological analysis 10 of specimen After 6 d in culture revealed the full spectrum of tubular differentiation including glomerulus formation.

As with spinal cord mediated induction, Wnt-4 expression 15 in the mesenchyme itself is not required for tubule formation, but supplying Wnt-4 in adjacent cells is sufficient to trigger the inductive process. These results suggest that whereas Wnt-4 plays an essential role in initial tubulogenesis, it may not be required for later morphogenesis of the tubule. As shown in Table 5, 20 Wnt-1 expressing cells were also sufficient to trigger tubulogenesis in mesenchyme mutant for Wnt-4.

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Table 5: Induction of tubulogenesis in Wnt-4/Wnt-4 mutant metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 or Wnt-1

#Exp	# Recombinants	#induced/#total
5	+ / +	Wnt-4 / + Wnt-4 / Wnt-4
with NIH3T3 cells expressing Wnt-4:		
4	42	7 / 7 18 / 18 17 / 17
with NIH3T3 cells expressing Wnt-1:		
10	20	5 / 5 11 / 12 3 / 3

Mammalian kidney development

Metanephric development is a highly coordinated process characterized by a continuous interaction of the epithelial ureter and the surrounding metanephric mesenchyme. Classical organ culture experiments have pointed to the fact that these two compartments achieve coordinated development by use of reciprocal signaling systems. First, the metanephric blastema induces a bud from the adjacent nephric duct which invades and branches into the mesenchyme. This process appears to be mediated by GDNF which is secreted by the metanephric mesenchyme and sensed by the c-ret/GDNFR α receptor complex on the ureter. Next, the metanephric mesenchyme undergoes tubulogenesis upon a permissive stimulus from the ureter.

Signals required for induction of tubulogenesis

In addition to Wnt-4, other Wnts may replace Wnt-4 activity in the mesenchyme. Using cell lines expressing various Wnt genes, Wnt-1, Wnt-3a, Wnt-7a, Wnt-7b, were

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shown to evoke tubulogenesis in isolated metanephric mesenchyme. The results described herein suggest a different interpretation of the use of kidney cultures to elucidate the nature of the ureteric signal involved in 5 inducing the mesenchyme. Experiments which have used heterologous sources of tubule inducers, e.g., the spinal cord, may not have been investigating the nature of ureteric signaling, but rather the mesenchymal action of signals such as Wnt-4. At present, the exact nature of 10 ureteric signaling remains obscure. A primary signal might be required for a sufficient length of time to allow auto-induction of the mesenchyme by Wnt-4. Alternatively, a secondary signal from the ureter tip might be necessary to induce Wnt-4 expression in 15 aggregating mesenchyme. In contrast to earlier studies, the data presented herein indicate that Wnt-11 does not play a role as a ureteric signal for mesenchymal aggregation. In the present studies, tubulogenesis was not detected with cells expressing Wnt-11.

20 Wnt-4 is a mesenchymal signal for tubulogenesis

Analysis of Wnt-4 mutants has demonstrated a critical role for Wnt-4 in kidney development. Homozygous pups die 24 h after birth due to small agenic kidneys consisting of undifferentiated mesenchyme 25 intermingled with collecting duct tissue. Histological and marker analysis revealed that primary condensation of mesenchymal cells around the ureter tips as well as ureteric branching occurs normally. However, mutant kidneys quickly become growth retarded and the mesenchyme 30 remains undifferentiated lacking pretubular cell aggregates and epithelial tubules. Since kidney size as well as cell death initially remain unaffected, proliferation is unlikely to be controlled by Wnt-4. Rather, the lack of Wnt-4 expression itself and of 35 epithelial structures in the mutant mesenchyme indicates

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that Wnt-4 may autoinduce the epithelialization of condensed mesenchyme. Mesenchymally-derived Wnt-4 is not only required but also sufficient for induction of tubulogenesis in the mammalian kidney. Judging by 5 histological and molecular markers, Wnt-4 can elicit the complete program of tubular differentiation in isolated metanephric mesenchyme. The activity of Wnt-4 contrasts with other factors thought to regulate mesenchymal development. For example, basic fibroblast growth factor 10 (FGF) and epidermal growth factor (EGF) can both support mesenchymal survival but are not sufficient for tubulogenesis. Like Wnt-4, BMP-7 has been suggested to induce tubules, but loss-of-function studies indicate it is not essential for tubule formation in vivo as some 15 glomeruli form in BMP7 mutants. In contrast, loss of Wnt-4 led to a complete absence of glomeruli.

Wnt-4 activity shows all the characteristics which have previously been ascribed to induction by dorsal spinal cord tissue. Signaling is cell-contact dependent. 20 Below a certain pore size in the transfilter assay the formation of cellular processes which penetrate the filter pores is inhibited and isolated mesenchyme degenerates. Cell contact is required for induction of tubulogenesis, and Wnt proteins may interact with 25 extracellular matrix (ECM) components.

Wnt-4 expression in the metanephric mesenchyme is initiated in the aggregating mesenchyme and maintained in the comma shaped bodies before it is downregulated in S-shaped bodies. Therefore, Wnt-4 likely has a later 30 function in tubulogenesis.

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Table 15: Human Wnt-4-encoding nucleic acid

1 TGCAAGTGTC ACGGGGTGTC AGGCTCCTGT GAGGTAAAGA CGTGCTGGCG
51 AGCCGTGCCG CCCTTCCGCC AGGTGGGTCA CGCACTGAAG GAGAAGTTTG
101 ATGGTGCCAC TGAGGTGGAG CCACGCCGCG TGGGCTCCTC CAGGGCACTG
151 GTGCCACGCA ACGCACAGTT CAAGCCGCAC ACAGATGAGG ACCTGGTGTA
201 CTTGGAGCCT AGCCCCGACT TCTGTGAGCA GGACATGCGC AGCGGGCGTGC
251 TGGGCACGAG GGGCCGCACA TGCAACAAGA CGTCCAAGGC CATCGACGGC
301 TGTGAGCTGC TGTGCTGTGG CCGCGGCTTC CACACGGCGC AGGTGGAGCT
351 GGCTGAACGC TGCAGCTGCA AATTCCACTG GTGCTTGTTC TTGAGTCGAC

SEQ ID NO: 10

Table 16: Human Wnt-7a-encoding nucleic acid

1 TGTAAGTGTC ACGGC GTGTC AGGCTCGTGC ACCACCAAGA CGTGCTGGAC
51 CACACTGCCA CAGTTTCGGG AGCTGGGCTA CGTGCTCAAG GACAAGTACA
101 ACGAGGCCGT TCACGTGGAG CCTGTGCGTG CCAGCCGCAA CAAGCGGGCCC
151 ACCTTCCCTGA AGATCAAGAA GCCACTGTGCG TACCGCAAGC CCATGGACAC
201 GGACCTGGTG TACATCGAGA AGTCGCCAA CTACTGCGAG GGGGACCCGG
251 TGACCGGCAG TGTGGGCACC CAGGGCCGCG CCTGCAACAA GACGGCTCCC
301 CAGGCCAGCG GCTGTGACCT CATGTGCTGT GGGCGTGGCT ACAACACCCA
351 CCAGTACGCC CGCGTGTGGC AGTGCAATTG TAAGTTCCAT TGGTGC

SEQ ID NO: 11

- 43 -

Table 17: Human Wnt-7b-encoding nucleic acid

1 GTAAAATGTC ACGGCGTGTC TGGCTCCTGC ACCACCAAAA CCTGCTGGAC
51 CACGCTGCCA AAGTTCCGAG AGGTGGGCCA CCTGCTGAAG GAGAAGTACA
101 ACGCGGCCGT GCAGGTGGAG GTGGTGCAGG CCAGCCGTCT GCGGCAGCCC
151 ACCTTCCCTGC GCATCAAACA GCTGCGCAGC TATCAGAAGC CCATGGAGAC
201 AGACCTGGTG TACATTGAGA AGTCGCCCAA CTACTGCGAG GAGGACGCGG
251 CCACGGGCAG CGTGGGCACG CAGGGCCGTC TCTGCAACCG CACGTCGCC
301 GGCGGGGACG GCTGTGACAC CATGTGCTGC GGCGAGGCT ACAACACCCA
351 CCAGTACACC AAGGTGTGGC AGTGCAACTG CAAATTCCAC TGGTGCTGC
401 CTAG

SEQ ID NO: 12

- 44 -

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the 5 invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

- 45 -

1. A method of stimulating kidney tubule formation in a post-natal mammal, comprising administering to said mammal a substantially pure Wnt polypeptide or a Wnt agonist, wherein said Wnt polypeptide is not Wnt-11.
2. The method of claim 1, wherein said mammal is characterized as suffering from a kidney disorder.
3. The method of claim 1, wherein said mammal is an adult mammal.
- 10 4. The method of claim 2, wherein said disorder is chronic renal failure.
5. The method of claim 2, wherein said disorder is renal cell carcinoma.
- 15 5. The method of claim 2, wherein said disorder is polycystic kidney disease.
6. The method of claim 2, wherein said disorder is chronic obstructive uropathy.
7. The method of claim 2, wherein said disorder is virus-induced nephropathy.
- 20 8. The method of claim 7, wherein said virus is HIV-1.
9. The method of claim 1, wherein said Wnt polypeptide is a Wnt-1 class polypeptide.

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10. The method of claim 1, wherein said Wnt polypeptide is selected from the group consisting of Wnt-3a, Wnt-4, Wnt-7a, and Wnt-7b.

11. The method of claim 1, wherein said Wnt polypeptide is Wnt-4.

12. The method of claim 1, wherein said Wnt agonist is HLDAT86.

13. The method of claim 1, further comprising administering a sulfated glycosaminoglycan.

10 14. The method of claim 1, wherein said Wnt polypeptide or Wnt agonist is administered locally to a renal tissue.

15 15. The method of claim 14, wherein said Wnt polypeptide or Wnt agonist administered by retrograde perfusion of said renal tissue.

16. The method of claim 1, wherein said Wnt polypeptide or Wnt agonist is administered *ex vivo* to an explanted renal tissue.

17. The method of claim 1, wherein said Wnt 20 agonist is a peptide mimetic.

18. The method of claim 1, wherein said Wnt polypeptide has an amino acid sequence at least 85% identical to SEQ ID NO:1, 2, 3, 4, or 5, and wherein said Wnt polypeptide induces tubulogenesis.

- 47 -

19. A method of stimulating kidney tubule formation in a post-natal mammal, comprising administering to said mammal a substantially pure nucleic acid encoding a Wnt polypeptide or a Wnt agonist.

5 20. An *ex vivo* mammalian kidney comprising an substantially pure exogenous Wnt polypeptide.

PCT

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International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US99/07745 (22) International Filing Date: 8 April 1999 (08.04.99)		(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(71) Applicant (for all designated States except US): PRESIDENT AND FELLOWS OF HARVARD COLLEGE [US/US]; 17 Quincy Street, Cambridge, MA 02138 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): MCMAHON, Andrew, P. [GB/US]; 128 Kendall Road, Lexington, MA 02173 (US). KISPERT, Andreas [DE/DE]; Lindenweg 12, D-74838 Limbach (DE). VAINIO, Seppo [FI/FI]; Tohointie 6, FIN-90570 Oulu (FI). (74) Agent: BEATTIE, Ingrid, A.; Mintz, Levin, Cohn, Ferris, Glovsky & Popeo PC, One Financial Center, Boston, MA 02111 (US).		

(54) Title: INDUCTION OF KIDNEY TUBULE FORMATION

(57) Abstract

The invention provides a method of stimulating kidney tubule formation in a post-natal mammal by administering to the mammal a substantially pure Wnt polypeptide or Wnt agonist.

Attorney Docket No. 21508-033 Natl

**COMBINED DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor which is claimed and for which a utility patent is sought on the invention entitled:

INDUCTION OF KIDNEY TUBULE FORMATION

the specification of which:

was filed on **September 28, 2001**, as United States non-provisional application **09/937,735**, bearing Attorney Docket No. **21508-033 Natl**.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application designating at least one country other than the United States listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

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PCT/US99/07745	April 8, 1999	Pending

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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H-00
APM

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Date

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Full Name of Inventor: Andreas Kispert

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Residence: Lindenweg 12, D-74838, Limbach, Germany

Post Office Address: Lindenweg 12, D-74838, Limbach, Germany

Inventor's Signature

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Full Name of Inventor: Seppo Vainio

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Post Office Address: Tohtorintie 6, FIN-900570 Oulu, Finland

TRA 1624973v1

Attorney Docket No. 21508-033 Natl

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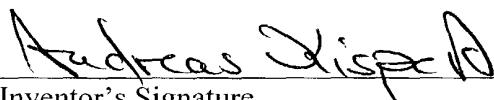
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Post Office Address: 128 Kendall Road, Lexington, MA 02173



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27. March 2002

Date

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Date

Full Name of Inventor: Seppo Vainio

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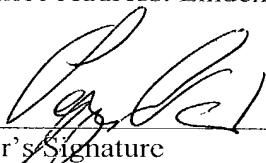
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TRA 1624973v1

SEQUENCE LISTING

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Kispert, Andreas
Vainio, Seppo

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<141> 2001-09-28

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<212> DNA
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<400> 12
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